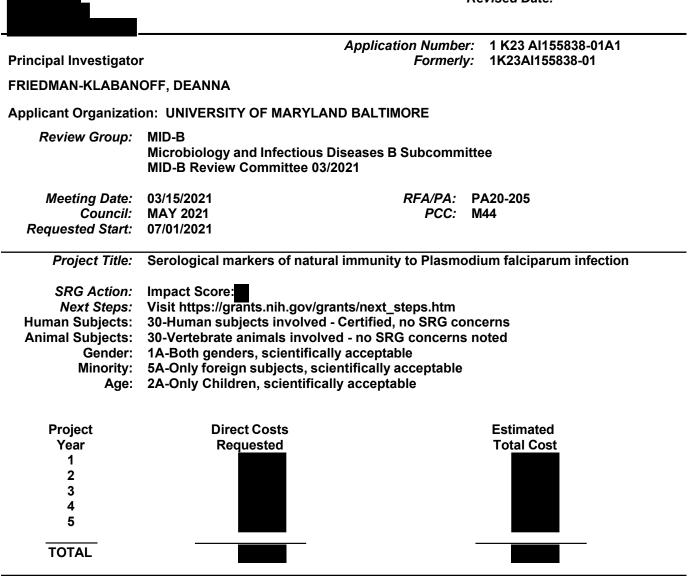
SUMMARY STATEMENT

(Privileged Communication)

PROGRAM CONTACT:

Release Date: 04/06/2021 Revised Date:



ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

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MID-B

1K23Al155838-01A1 Friedman-Klabanoff, D

FOREIGN INSTITUTION

RESUME AND SUMMARY OF DISCUSSION: This exceptional resubmission of a Mentored Patient-Oriented Research Career Development Award application entitled "Serological markers of natural immunity to Plasmodium falciparum infection" was submitted in response to PA20-205 (PARENT K23) by the University Of Maryland, Baltimore with DeAnna Friedman-Klabanoff, MD, as Principal Investigator (PI).

The goal of this revised application is to provide the candidate with a training experience in patientoriented research evaluating samples using sera from malaria-exposed children in an ongoing cohort study begun in 2019 in Malawi. The work will proceed according to the following specific aims: Aim 1: Identify serologic responses associated with natural protection against *P. falciparum* infection. Aim 2: Assess the function of antibodies targeting *P. falciparum* pre-erythrocytic antigens of interest.

This resubmitted application has many strengths and virtually no significant weaknesses. The candidate as in the original application is extremely well qualified with multiple honors, impressive publications and laudatory references. The project is significant as it relates to malaria vaccine development using *in silico* and *in vitro* analyses of antibody response to P. falciparum epitopes using a unique human cohort. Access to this Malawian cohort is a major strength. The candidate has addressed the major issues of the original submission with more detail about the cohort - the University of Malawi College of Medicine and the Malaria Alert Center. The candidate has assembled an expert mentoring team and has the facilities and resources of major institutions. The panel agreed that this application was responsive to the issues raised in the prior review and is now at an exceptional level. Based upon the evaluation of scientific and technical merit, this application received an Impact/Priority score of

DESCRIPTION (provided by applicant): Plasmodium falciparum is the most common and deadly cause of malaria. An effective malaria vaccine has the potential to make a pivotal change in malaria control and eradication. For a vaccine to contribute significantly to malaria eradication, it must target the early, pre-erythrocytic part of the lifecycle to block both symptomatic disease and asymptomatic infection, which perpetuates transmission. Naturally acquired immunity to pre-erythrocytic infection is acquired with exposure but remains poorly understood and continues to impede vaccine efforts. DeAnna Friedman-Klabanoff, M.D., a pediatric infectious disease specialist at the University of Maryland School of Medicine, developed this career development award proposal to use novel highthroughput tools to define naturally acquired humoral immunity to diverse pre-erythrocytic epitopes associated with protection, which could lead to novel vaccine candidates. Dr. Friedman-Klabanoffs long-term goal is to become an independent clinical and translational researcher dedicated to the development of a malaria vaccine, applying immunology and data science to inform and optimize vaccine development. To gain the skills necessary to achieve this goal, Dr. Friedman-Klabanoff proposes a career development plan that includes mentoring from Drs. Miriam Laufer, Shannon Takala Harrison, Michael Cummings, Andrea Berry, Kathleen Neuzil, and John Adams, leaders in the fields of international research design and leadership, molecular epidemiology, data science for analysis of large data sets, use of peptide microarrays to study malaria, vaccinology, and in vitro models of preerythrocytic immunity. This project will utilize samples and data from a cohort study of malaria in Malawi led by Dr. Laufer, the primary mentor for this proposal. Household members were followed monthly for detection of malaria infection and mosquitoes were collected from the houses to identify bloodmeal

sources. Bloodmeal sources will be identified by matching the human DNA found in the mosquito bloodmeals to DNA from enrolled participants . Mosquito salivary glands will also be tested for P. falciparum infection to determine if the mosquitoes were infectious. Children will be defined as protected or infected based on whether they develop blood-stage infection during the month after an infectious bite. Aim 1 of this proposal will be to identify serologic responses associated with natural protection against P. falciparum infection after exposure to an infectious bite. Serum from the day of exposure will be probed on a custom-developed peptide microarray designed from diverse, field-derived sequences to characterize pre-exposure immunity to pre-erythrocytic antigens. Aim 2 of this proposal is to assess the functional role of antibodies targeting P. falciparum pre-erythrocytic antigens of interest. B- and T- cell epitope prediction tools will be used to find predicted epitopes in pre-erythrocytic proteins and their variants, and in vitro liver models will be used to assess the functional role of antibodies target and down select the potential epitopes. The practical implications of this work will be to identify promising epitopes that are targets of protective immunity.

PUBLIC HEALTH RELEVANCE: Plasmodium falciparum, the most common species of malaria, kills more children than any other pathogen, and the development of a highly effective vaccine has been challenging. Children acquire immunity to malaria with repeated exposure, but the mechanism is poorly understood. We will collect samples from children who are exposed to Plasmodium-infected mosquito bites and compare the immune responses among those who develop infection to those who do not to identify possible targets for vaccines to prevent malaria infection.

CRITIQUE: The comments in the CRITIQUE section were prepared by the reviewers assigned to this application and are provided without significant modification or editing by staff. They are included to indicate the range of comments made during the discussion, and may not reflect the final outcome. The RESUME AND SUMMARY OF DISCUSSION section summarizes the final opinion of the committee after the discussion and is the basis for the assigned Overall Impact/Priority score.

CRITIQUE 1

Candidate: Career Development Plan/Career Goals /Plan to Provide Mentoring: Research Plan: Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): Environment Commitment to the Candidate:

Overall Impact: This application is a revised K23 from a well-trained physician with an outstanding mentoring team. The application is well-designed to build on the candidate's prior experience and will help her reach her long-term career goal, to become an independent clinical and translational researcher dedicated to malaria vaccine development. The revised application was very responsive to the previous critiques by adding an immunology mentor John Adams, including time to work in his lab in Florida to learn the in vitro liver infection model, machine learning to analyze the peptides that elicit protection, and many controls for the research strategy. The candidate has received several awards

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and has an approved publication record with 3 first author publication in the past 3 years. Excellent mentoring team that cover all the necessary aspects of this application. Well written mentoring letters, especially from Dr. Miriam Laufer, which detail the weekly and biweekly meeting with the mentor and co-mentors, respectively, as well as guarterly meetings with her advisory committee. Outstanding environment and facilities for the proposed studies at the University of Maryland with the Center for Vaccine Development and Global Health. In response to the previous critiques, information was added about the excellent facilities at the University of Malawi College of Medicine and the Malaria Alert Center. Detailed career development plan that defines 5 short term goals to achieve during this award cycle, which will help the candidate progress towards her long-term goal of becoming and independent investigator that combines clinical and translational research. The goal of the research proposal is to use high throughout tools to characterize the natural protective antibody response to the preerythrocytic stages of P. falciparum in children in Malawi. Understanding the epitopes that are protective during natural infection will aid in the design of future vaccines. The first aim will analyze a unique set of sera, sampled over time from Malawi children who did or did not present with malaria, on a peptide array with up to 392,000 P. falciparum peptides. The functionality of antibodies directed against these epitopes will be tested in the second aim using an in vitro liver model from the Adams lab. While there were a few weaknesses noted with the research proposal, overall this was a greatly improved application from an exceptionally strong applicant and mentoring team, so enthusiasm was high.

1. Candidate:

Strengths

- Well-trained physician that has had a variety of training experiences that have prepared her for these studies. In addition to, her medicine residency and Peds ID fellowship, she did clinical training in Rwanda and is completing a postdoctoral fellowship for additional research training.
- The candidate has received several awards including the BWF/Trop Med postdoctoral fellowship and Pichichero Family Research development award.
- The candidate has an improved publication record with 3 first author publication in the past 3 years. An exceptionally strong record given her clinical duties.
- Strong references letters that attest to this well-trained physician being a caring physician and becoming an outstanding independent investigator

Weaknesses

• None were noted.

2. Career Development Plan/Career Goals and Objectives:

- The candidate's prior training and research experience provide an excellent background for her to perform the proposed studies.
- The candidate has 5 defined and obtainable short term goals to achieve during this award cycle, that will help her progress towards her long term goal of becoming and independent investigator that combines clinical and translational research.
- Excellent plan for meeting with mentoring on a regular basis to monitor the candidate progress.

Weaknesses

• None were noted.

3. Research Plan:

Strengths

- From high transmission areas in southern Malawi, this application will analyze mosquitoes and blood samples from Plasmodium-exposed children to character naturally acquired humoral immune responses to pre-blood stage epitopes.
- The research proposal is highly significant and will examine methods to distinguish protection from lack of exposure, correlates of protection, and genetic diversity of P. falciparum. Very relevant to the candidates stated career goals.
- Results from this application will build toward a future R01 for the candidate to further characterize identified epitopes of interest using in vitro and in vivo functional studies to select novel malaria vaccine candidates.
- Strong preliminary data using an innovative >170,000 peptide microarray to quantify antibody
 responses to AMA1 vaccination. Assuming this blood samples was a finger prick, it shows they
 will have enough blood from the children to do the proposed studies.
- In response to the previous critiques, excellent addition of John Adams and the in vitro liver model as well as machine learning algorithms, such as Random Forest, to identify which peptide(s) best predict infection and protection.
- Adequate rigor and addressing of potential obstacles and biological variables for the human subjects. Sample sizes seem to have adequate power.

Weaknesses

- How predictive is the in silico model? Is the protection only antibody-mediated?
- How does knowing when the child was infected characterize the genetic diversity of P. falciparum? Unclear what will be learned from the mosquito experiments.
- Is matching 25 of the 28 mosquitoes with their blood meal source good enough? Looks like it was done only once so is that reproducible? How will they determine that the children are the "sole source" of the blood meal.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

- Dr. Miriam Laufer is an outstanding mentor for the proposed studies. She has an outstanding mentoring, publication and funding record.
- Dr. Harrison will be an excellent co-mentor for this physician mentored award so she is a physician-scientist with an excellent funding and publication record.
- Excellent advisory committee with Drs. Neuzil and Berry. The candidate already has 2 first author publications with Dr. Berry, showing that they have active and productive mentoring relationship.
- Excellent addition of malaria immunologist Dr. John Adams in response to the previous critiques. He is a leader in the malaria vaccine field. The candidate will spend two months training in the Adams laboratory, which is an excellent addition.

• Well written mentoring letters, which details the weekly and biweekly meeting with the mentor and co-mentors, respectively as well as quarterly meetings with her advisory committee.

Weaknesses

None

5. Environment & Institutional Commitment to the Candidate:

Strengths

- Outstanding environment and facilities for the proposed studies at the University of Maryland with the Center for Vaccine Development and Global Health.
- In response to the previous critiques, information was added about the excellent facilities at the University of Malawi College of Medicine and the Malaria Alert Center. The equipment and space needed for this research project are in place.
- Dr. Adams lab has outstanding facilities for the 2 months of training that the candidate will have in his lab.
- Strong letter of support from the department chairs saying that she will have at least 75% protected time for this research project.

Weaknesses

• None were noted.

CRITIQUE 2

Candidate:	
Career Development	
Plan/Career Goals /Plan to	
Provide Mentoring:	
Research Plan:	
Mentor(s), Co-Mentor(s),	
Consultant(s),	
Collaborator(s):	
Environment Commitment	
to the Candidate:	

Overall Impact: This is a resubmission. P. falciparum is the most common cause of malaria. In this study, this application aims to use novel high-throughput tools to characterize naturally acquired humoral immunity to diverse pre-erythrocytic epitopes, with an ultimate goal of developing an effective vaccine against Plasmodium falciparum, the most common and deadly cause of malaria. The candidate is well trained with clinical and basic research. Two aims are proposed. Aim 1 is more experimental and targets to identify serological responses associated with natural protection against P. falciparum infection. Aim 2 is more in silico analysis to identify functional B- and T-cell epitopes. A novelty is the usage of the real field samples since most studies do not. Overall the experimental design contains many novel components and methods. The applicant has effectively addressed the comments provided in last submission.

1. Candidate:

Strengths

- The candidate is well trained with both clinical and basic science research. She had a B.S. in 2004 in medical microbiology and immunology, her MD in 2009, a Pediatrics Residency until 2012, and a fellow in pediatric infectious diseases until 2019.
- The candidate had postdoctoral fellowship in University of Maryland School of Medicine, Baltimore, until July 2020.
- She is currently a pediatric hospitalist, and an instructor, University of Maryland School of Medicine, Baltimore, MD.
- She has authored 6 (4 at the first author) peer-reviewed articles, including 1 in 2020, 2 in 2019, 1 in 2016, 1 in 2012, and 1 in 2011. Two out of 3 papers since 2019 are first-author papers.
- She is currently PI for two grants.

Weaknesses

None

2. Career Development Plan/ Career Goals & Objectives:

Strengths

- The candidate's long-term research goal is to become an independent investigator dedicated to developing a highly efficacious malaria vaccine. This project will help her achieve the long term goal.
- The short term goals including various trainings international project management, computational biology, machine learning, biostatistics, academic skills such as grant writing and leadership skills.
- Formal courses with credits will be taken.

Weaknesses

• None.

3. Research Plan:

- Individual level exposure to malaria in a field setting.
- Well-designed sample collection and processing. Samples will be collected as part of the community-based household surveillance cohort from Malawi. The mosquitoes will be collected, and the human DNA from the human blood in the mosquito midgut will be recovered. The human source of the identified blood will be defined using human microsatellite technology. Whether the mosquito is infected will be detected by PCR using salivary glands of the mosquitoes. These techniques have been successfully applied.
- A unique peptide array that contains P. falciparum antigens of interest and their naturally occurring genetic variants will be designed and applied.
- Aim 1 is to identify serological responses associated with natural protection against P. falciparum infection. The hypothesis is that sera from malaria-exposed children who are protected from blood-stage P. falciparum infection will recognize pre-erythrocytic epitopes, but they will not be recognized by children who develop blood-stage infection.

- Aim 2 is more in silico to identify functional B- and T-cell epitopes. The state-of-the-art in silico analysis methods will be used.
- The PI has addressed many comments from the reviewer. More pitfalls and alternative solutions are provided.

Weaknesses

- Unlike T cell epitope prediction, the prediction accuracy of antibody epitopes is still not ideal. It appears that only linear protective epitopes will be identified.
- With the serological focus, the role of cell-mediated immunity will not be characterized. Humoral immunity is known to be important in protection against malaria; however, cell-mediated immunity may also play an important role. The PI proposes it to be done for future work.
- It would be good to experimentally verify some in silico epitope predictions in Aim 2, which may be done in the future.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths

- The mentoring team is strong.
- Dr. Miriam Laufer, Director of the Malaria Research Program (MRP) at the CVD, is the primary mentor. Dr. Laufer is an expert in malaria epidemiology and elimination.
- Dr. Shannon Takala Harrison, Head of the Genomic Epidemiology Unit in MRP at the CVD, will serve as a co-mentor. Dr. Harrison is an expert in understanding how malaria parasite diversity affects drug resistance and vaccine effectiveness.
- Dr. Michael Cummings, Director of the Center for Bioinformatics and Computational Biology, will also serve as a co-mentor.
- Dr. John Adams, a recognized immunologist in the Plasmodium immunology, has been added as an advisor.
- The advisory committee also includes Dr. Kathleen Neuzil (expert in vaccinology) and Dr. Andrea Berry (expert in Immunoepidemiology and Pathogenesis).

Weaknesses

• None.

5. Environment and Institutional Commitment to the Candidate:

Strengths

- The Center for Vaccine Development (CVD) in the University of Maryland provides an ideal environment for the proposed research.
- The institutional commitment is very good.

Weaknesses

• Not observed.

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Candidate: Career Development Plan/Career Goals /Plan to Provide Mentoring: Research Plan: Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): Environment Commitment to the Candidate:

Overall Impact: This is an outstanding resubmission of an application from an outstanding candidate for a K23 career development award. For the outlined career goals and long-term plans for research in the field of malaria there is not likely a more supportive and enhancing environment than the University of Maryland School of Medicine and its affiliated research centers on malaria, vaccines, and bioinformatics. The resubmission includes the provision of additional expertise in immunology and now documents the features of the research site in Malawi. The research plan overall is appropriate for the career goals and achieving hands-on training. Minor weaknesses of the original application have been convincingly addressed.

1. Candidate:

Strengths

- Outstanding physician-scientist candidate for the outlined training and type of research project. The applicant's background and research accomplishments to date provide the foundation for these next steps.
- Microbiology research began as an undergraduate and global health experiences in Africa began during medical school.
- Excellent clinical training and research experiences to date after medical school.
- A weakness in the first application has been eliminated. There are now two published first author papers--one in Journal of Infectious Diseases and one in Vaccine--relevant to the proposed project.

Weaknesses

None

2. Career Development Plan/Career Goals and Objectives:

Strengths

- Coursework is appropriate for career goals and are not duplicative of past training.
- Research project complements the didactic material and the one-on-one mentoring.
- Will get a Master of Science in Clinical Research.
- Professional career development opportunities that the applicant will make use of at the University of Maryland School of Medicine are outstanding.

Weaknesses

None

3. Research Plan:

Strengths

- The applicant and mentors have identified an important area of study both from the basic science view and global health view.
- Although there are limitations to what the peptide array can reveal, the data will likely fill in some of the missing pieces with regard to naturally-aquired immunity. The results will be informative for further vaccine efforts as well as epidemiologic studies.
- The results from the peptide study could lead to second generation assays based on a smaller number of peptides and consequently of lower cost and complexity.
- The research project extends the studies the applicant is currently, is part of a larger effort from which there is large capacity of support, and will utilize an infrastructure in Africa that is well-developed and epidemiology of malaria that is well-characterized.
- A power analysis was included. Although there was no attempt to include stratification (see below), it may be sufficient to justify a limited study.
- The research project will likely provide a training experience that will complement the other skills and expertise the applicant has already demonstrated.
- The application of machine learning and similar approaches to data analysis is justified for this complex data.
- Aim 2 is dependent to some extent on Aim 1 findings, but it is focused and straight-forward. Should provide the applicant with a broader background in immunology as well.
- In the resubmission there is better documentation of the assessments of immunity using the arrays and epitope prediction programs.
- The resubmission includes more details about the machine learning procedure and describes replication using training and testing sets.
- There is a more extended discussion of the strengths and limitations of the peptide arrays for evaluating immunity. The applicant outlines alternative assessments that could be done in the future to complement the arrays.
- In the resubmission there is a more compelling plan to evaluate candidate antigens from the arrays using antisera raised in rabbits in in vitro functional models and an inhibition of liver stage development assay.

Weaknesses

None

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

- Miriam Laufer, MD, MPH is Professor of Pediatrics and Director of the Center for Vaccine Development and Global Health at the University of Maryland School of Medicine. PI on D43 Fogarty training grant for graduate training in biostatistics, vector biology, and molecular epidemiology
- Shannon Takala Harrison, PhD is Associate Professor of Medicine and of Epidemiology and Public Health at the University of Maryland School of Medicine. Leads the molecular

epidemiology track with the doctoral program. Experience in mentoring graduate students. Wellqualified to provide mentorship and advice for epidemiology and specifically molecular epidemiology and population genetics

- Michael Cummings, PhD is Professor of Biology and Director, Center for Bioinformatics and Computational Biology at University of Maryland College Park. Well-qualified to provide mentorship and advice for computer science (specifically machine learning), bioinformatics, and evolutionary biology aspects of the research project and training.
- Another member of the mentoring committee is Kathleen Neuzil, MD, MPH, endowed Professor of Vaccinology and Professor of Medicine and Pediatrics, as well as Director of the Center for Vaccine Development and Global Health at the University of Maryland. Although a biosketch was not included, Professor Neuzil appears to be well-qualified to serve on the mentoring committee.
- Another advisor is Andrea Berry, M.D., Assistant Professor of Pediatrics and Medicine, who is co-director of the Immunoepidemiology and Pathogenesis Unit within the Malaria Research Program at the University of Maryland. Dr. Berry has expertise in protein and peptide array technologies and their application for vaccine and epidemiology studies. Dr. Berry worked closely with candidate on a study that involved peptide array and that has been submitted for publication. The resubmission includes of biosketch for Dr. Berry as well as a letter.
- The resubmission is strengthened by the addition Professor John Adams of the University of South Florida as an adviser and provider of training for the applicant in the use of in vitro to study pre-erythrocytic immunity to Plasmodium spp.

Weaknesses

• None.

5. Environment & Institutional Commitment to the Candidate:

Strengths

- Outstanding environment at the University of Maryland for training and research in the proposed areas of the career development plan.
- Specific intent to appoint the candidate to the faculty at the Instructor level. This appointment and accompanying protected research time is not contingent on obtaining this award.
- Institution commits to at least 75% time for research.
- The resubmission includes much more information about the study site in Malawi and the affiliated institutions.

Weaknesses

None

Resubmissions:

Comments or Concerns: Acceptable. This is a much improved application. The candidate has addressed the concerns and comments of the reviewers.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: Code 30 ACCEPTABLE

This study involves characterization of humoral responses to *Plasmodium falciparum* before and after exposure to infectious bites of mosquitoes. The human and entomological samples for the proposed research will be collected as part of a Malawi International Center of Excellence in Malaria Research (ICEMR) Program cohort study that began early 2019.

INCLUSION OF WOMEN PLAN: Code G1A ACCEPTABLE

The study will recruit participants without regard to gender.

INCLUSION OF MINORITIES PLAN: Code M5A ACCEPTABLE

All participants will be Malawians of African descent.

INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN: Code C2A ACCEPTABLE

Samples for Aim 1 will be serum from children aged 6 months to 15 years eligible for the proposed research.

VERTEBRATE ANIMAL: Code 30 ACCEPTABLE

Female and male New Zealand white rabbits aged <1 year will be used for generation of antibodies.

BIOHAZARDS: ACCEPTABLE

Comments or Concerns: Revised application now addresses precautions taken when human blood and specimens. Adequate biohazard equipment is available in each research location.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH: ACCEPTABLE

Comments or Concerns: The candidate has taken several RCR and human subjects training courses. She will take an additional 1 credit RCR course in the spring of 2022 that covers all of the necessary topics.

Comments or Concerns: Acceptable. The resubmission includes a more detailed description of training taken and to be taken.

SELECT AGENT: NOT APPLICABLE

RESOURCE SHARING PLANS: ACCEPTABLE DATA SHARING PLAN: MODEL ORGANISMS SHARING PLAN: GENOMIC DATA SHARING PLAN:

FOREIGN INSTITUTION: JUSTIFIED

The human and entomological samples for the proposed research will be collected as part of a Malawi International Center of Excellence in Malaria Research (ICEMR) Program cohort study that began early 2019.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES: ACCEPTABLE

Comments: The revised authentication of reagents is acceptable and very detailed.

COMMITTEE BUDGET RECOMMENDATIONS: ACCEPTABLE

The budget is recommended as requested in all years.

Footnotes for 1 K23 AI155838-01A1; PI Name: Friedman-Klabanoff, DeAnna

NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see

http://grants.nih.gov/grants/peer_review_process.htm#scoring.